ORIGINAL ARTICLE

Cycads in the insular South-west Pacific: dispersal or vicariance?

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ABSTRACT

Aim Cycads constitute an ancient plant group that is generally believed to disperse poorly. However, one group of cycads (subsection Rumphiae) is thought to have dispersed relatively recently from a Malesian source area westwards to East Africa and eastwards into the Pacific, using a floatation-facilitating layer in their seeds. We use morphological and allozyme characters to investigate the relationships among the species within this group and to deduce whether the wide distribution was achieved by recent dispersal (as evidenced by high genetic similarity) or more distant vicariance events (high genetic differentiation).

Location We examined specimens collected throughout the range of subsection Rumphiae, from East Africa through Southeast Asia to Tonga in the South-west Pacific.

Methods We investigated relationships within subsection Rumphiae of the genus *Cycas* by analysing 18 variable (11 informative) morphological characters and 22 allozyme loci for seven of the 10 species currently assigned to this taxon.

Results Distinctive morphological characters are few and fail to resolve relationships within the group. Allozyme data show that species within this subsection are closely related and suggest that there are two groups within the subsection, one comprising *Cycas thouarsii* (East Africa) and *C. edentata* (the Philippines), and the other the remaining species (from Malesia and the Pacific). The Australian species *C. silvestris* is sister to subsection Rumphiae in the morphological analysis but is closely allied to *C. rumphii* (nested within the subsection) in the allozyme analysis, suggesting that Rumphiae may be paraphyletic and that characters thought to be taxonomically important may need to be re-evaluated.

Main conclusions Cycads within subsection Rumphiae are closely related, and the wide distribution of this group was probably achieved through relatively recent oceanic dispersal events. Separate events probably account for the dispersal of these cycads into the Pacific and to Africa. The origin and distribution of *C. silvestris* (Australia) could be explained by a dispersal event from New Guinea or may have resulted from a former land connection between Australia and New Guinea.

Keywords

Allozymes, cycads, Cycadaceae, *Cycas*, dispersal vs. vicariance, historical biogeography, Pacific, Rumphiae.

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INTRODUCTION

Cycads constitute an ancient group of slow-growing plants with about 300 extant species in 11 genera dating back some 250 Myr (Hill & Stevenson, 1998). Because some early fossils have great similarity to present-day species, they are often referred to as the 'dinosaurs of the plant world'. Whether this conservative morphology is related to slow evolutionary rates or genetic constraints is still debated. Most present-day species are restricted to relatively narrow geographic areas that are generally considered relics of previously wider distributions.

Cycas is the most widespread and diverse genus of cycads, with 98 species (Hill *et al.*, 2007) distributed from Africa eastwards to the Pacific islands and from China and southern Japan southwards to Australia, and is basal to all other extant cycads (Chaw *et al.*, 2005). Fossils assignable to the genus date back some 200 Myr (Norstog & Nicholls, 1997) and suggest that the morphology of this genus has changed little. This conservative morphological evolution (especially in vegetative features) has resulted in much taxonomic confusion (Hill, 1994a,b; Yang & Meerow, 1996). Subgeneric classification in *Cycas* has been especially problematic, and several alternative systems have been proposed (Schuster, 1932; Smitinand, 1971; Hill, 1995, 2004; Wang, 1996; de Laubenfels & Adema, 1998). All classifications agree in identifying megasporophyll shape (pectinate vs. non-pectinate) as taxonomically important, but they differ in the degree to which this character is emphasized in relation to other characters, such as leaf, stem, and ovule features (Table 1). Hill's (1995) treatment, updated by Hill & Stevenson (1998), is based on the most extensive study of both herbarium and living specimens (see also Hill, 1994a,b, 2004; Hill & Yang, 1999) and relies almost entirely on reproductive characters, an approach that has proved useful in other gymnosperm genera (Whitmore, 1980). Seed characters seem to be especially consistent and relevant for subgeneric classification (Hill, 2004).

Subsection Rumphiae represents a complex of closely related but widely distributed taxa, ranging from Southeast Asia and Malesia (an area that includes the Malay Peninsula, Indonesia, New Guinea and the Philippines) north to coastal South Indochina, south to New Guinea, as far west as East Africa, and as far east as Fiji and Tonga (Fig. 1). In the insular South-west Pacific (an area that includes non-continental Pacific islands south of the equator and east of New Guinea and Australia, extending to Samoa and Niue in the west), all native cycads have been placed in this subsection. In addition to the type species, *C. rumphii* Miq. (Malesia), the subsection includes *C. thouarsii* R. Br. ex Gaudich. (E Africa and Indian Ocean islands), *C. zeylanica* (J. Schust.) A. Lindstr. & K.D. Hill (Sri Lanka and Andaman Group), *C. litoralis* K.D. Hill (Thailand), *C. falcata* K.D. Hill (Sulawesi, Indonesia), *C. edentata* de Laub. (Philippines), *C. micronesica* K.D. Hill (Mariana Islands), *C. bougainvilleana* K.D. Hill (New Britain to Solomon Islands), and *C. seemannii* A. Braun (Vanuatu, New Caledonia, Fiji, and Tonga) (Hill & Stevenson, 1998; Hill, 1999, 2004; Lindström & Hill, 2002). The cultivated 'yellow cycad' (also known as *C. rumphii* f. *aurea* and in this paper referred to as *Cycas* 'Fiji Gold') is so called because of its yellow leaves when grown in full sunlight. This taxon may also belong to subsection Rumphiae (Hill, 1994b,c), but its taxonomic status remains uncertain

Table 1 Summary of taxonomic treatments of the genus *Cycas*.

Schuster (1932)	Smitinand (1971)	de Laubenfels & Adema (1998)	Hill (2004)
§ *Lemuricae:* non-pectinate megasporophylls	§ *Stangerioides:* subterranean caudex	**Genus *Epicycas:*** subterranean caudex	§ *Asiorientalis:* tomentose ovules
§ *Asiorientalis:* pectinate megasporophylls, tomentose ovules	§ *Cycas:* aerial caudex	**Genus *Cycas:*** aerial caudex	§ *Cycas:* glabrous ovules, non-pectinate megasporophylls
§ *Indosinenses:* pectinate megasporophylls, glabrous ovules	Sub§ *Circinnalidae:* non-pectinate megasporophylls	**Subgen. *Cycas:*** non-pectinate megasporophylls with triangular apex	§ *Panzhihuaenses:* glabrous ovules, pectinate megasporophylls, rigid and waxy male cones, microsporophyll apices deflexed, sclerotesta not ribbed
	Sub§ *Pectinatae:* pectinate megasporophylls	**Subgen. *Truncata:*** non-pectinate megasporophylls with truncate apex	§ *Wadeanae:* glabrous ovules, pectinate megasporophylls, rigid and waxy male cones, microsporophyll apices deflexed, sclerotesta ribbed
		Subgen. *Pectinata:* pectinate megasporophylls, midrib of adaxial pinnae surface prominent	§ *Indosinensis:* glabrous ovules, pectinate megasporophylls, soft male cones, microsporophylls rigid and acuminate with apices not deflexed; sarcotesta fibrous and smooth
		Subgen. *Revoluta:* pectinate megasporophylls, midrib of adaxial pinnae surface flat	§ *Stangerioides:* glabrous ovules, pectinate megasporophylls, soft male cones, microsporophylls flexible and rounded with apices not deflexed; sarcotesta not fibrous and verrucose

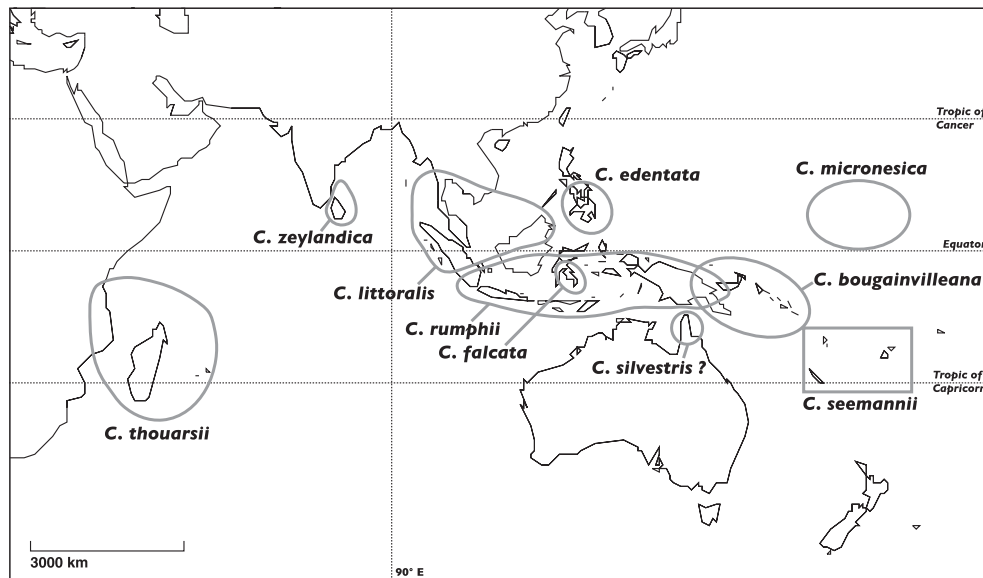§, section; sub§, subsection; Subgen, subgenus.

Figure 1 Distribution of the described species in *Cycas* subsection Rumphiae. *Cycas silvestris* is marked with a question mark because its affinity with the group has not yet been determined.

because natural populations have not yet been discovered, and because both seeds and male cones are unknown.

The general decrease in taxonomic diversity from Malesia eastwards is a well-documented phenomenon (Corner, 1963; van Balgooy, 1969; Woodroffe, 1987). The abundance of taxa lacking obvious dispersal traits and the presence of ancient lineages have led many to propose continental origins for both the flora and fauna of the Pacific islands (van Steenis, 1962; Thorne, 1963; Whitmore, 1969). However, the lack of obvious dispersal traits does not exclude the possibility of trans-oceanic dispersal (Jordan, 2001). The oceanic origin of most islands in the Pacific has now been firmly established (Yan & Kroenke, 1993; Hill, 2001). However, vicariance explanations have been considered the most likely scenario for conifers (de Laubenfels, 1996) and Proteaceae (Weston & Crisp, 1996) in the Pacific. Heads (2006) suggested that the young age of current Melanesian and Polynesian islands does not exclude the possibility of older, rafting continental fragments that are now largely submerged (as suggested for one New Caledonian fragment in Yan & Kroenke, 1993). Components of the biota of these continental fragments could have progressively moved to younger islands after their emergence through a stepping-stone model.

The presence of ancient lineages such as *Cycas* on remote islands in the Pacific is biogeographically interesting. Hill (1996) suggested that the distribution of *Cycas* is not relictual but rather resulted from recent and active dispersal made possible by a spongy layer in the seeds that confers positive buoyancy in seawater (Dehgan & Yuen, 1983; Keppel, 2001). This hypothesis largely agrees with the viewpoint of the importance of occasional long-distance dispersal events in Pacific biogeography that is currently popular in the literature (van Balgooy, 1969, 1996; Wright *et al.*, 2000; Winkworth *et al.*, 2002; Motley *et al.*, 2005) but contrasts strongly with

vicariance explanations, which propose that taxa arrived in the Pacific much earlier. It could also explain the presence of *Cycas rumphii* on the Krakatau Islands only 25 years after the volcanic eruption that sterilized them (Whittaker *et al.*, 1989).

The theory of recent, long-distance dispersal has received its strongest support from molecular data (Wright *et al.*, 2000; Winkworth *et al.*, 2002; Motley *et al.*, 2005). Pole (1994, 2001) even suggested that all or the great majority of the New Zealand flora resulted from this mechanism. The evidence is probably most convincing for Hawai'i, where the taxa show links (sometimes quite recent) with Africa, the Americas and Asia (e.g. DeJoode & Wendel, 1992; Seelanan *et al.*, 1997; Kim *et al.*, 1998; Lindqvist & Albert, 2002). Even the presence of the evolutionary old conifer genus *Agathis* in the insular Southwest Pacific, which was long considered an example of the Gondwanan connection of many Pacific islands (de Laubenfels, 1996), has been attributed to recent dispersal from New Caledonia (Setoguchi *et al.*, 1998).

Differences in the various taxonomic treatments of *Cycas* (Tables 1 and 2) illustrate the difficulties in the delimitation of subgeneric taxonomic units in this genus, making biogeographical inferences difficult. Similar taxonomic confusion exists in other cycad genera, such as *Zamia*, *Encephalartos* and *Macrozamia* (Newell, 1989; Vorster, 1993; Sharma *et al.*, 1998). Molecular approaches to phylogeny reconstruction have helped solve many biogeographical (DeJoode & Wendel, 1992; Setoguchi *et al.*, 1998; Wright *et al.*, 2000) and taxonomic riddles (Bremer *et al.*, 1995; Knox & Palmer, 1995; Soltis *et al.*, 2005), and allozymes have proved particularly useful for comparing taxa within and among closely related genera in many plant groups (see Crawford, 1983, 1989; Weeden & Wendel, 1989), including the cycad genus *Encephalartos* (van der Bank *et al.*, 1998). We therefore use molecular data combined with morphological characters gathered from

Table 2 Comparison of two classification systems for the *Cycas* species in which the megasporophylls are non-pectinate.

de Laubenfels & Adema (1998)	Hill (2004)
***Cycas* subgenus *Cycas*:**	***Cycas* section *Cycas*:**
Cycas thouarsii	**Subsection *Cycas*:**
***Cycas* subgenus *Truncata*:**	*Cycas scratchleyana*
Cycas bougaivilleana	**Subsection *Endemicae*:**
(as syn. of *C. rumphii*)	*Cycas silvestris*
Cycas edentata	**Subsection *Rumphiae*:**
Cycas micronesica	*Cycas bougaivilleana*
(as syn. of *C. celebica*)	*Cycas edentata*
Cycas rumphii	*Cycas falcata*†
Cycas scratchleyana	*Cycas litoralis*†
Cycas seemannii	*Cycas micronesica*
(as syn. of *C. celebica*)	*Cycas rumphii*
Cycas silvestris	*Cycas seemannii*
Not treated:	*Cycas thouarsii*
Cycas falcata (described in 1999)†	*Cycas zeylandica*†
Cycas litoralis (described in 1999)†	*Cycas* 'Fiji Gold'*
Cycas zeylandica (described in 2002)†	
Cycas 'Fiji Gold' (not a described species)	

*Placement of *Cycas* 'Fiji Gold' according to Hill (1994c).
†Species not sampled in the present study.

several species of subsection Rumphiae: (1) to explore the utility of allozyme data in determining evolutionary relationships among morphologically similar species in the genus *Cycas*; (2) to determine whether molecular data support Hill's (1996) hypothesis that the subsection originated from a Southeast Asian/Malesian source area, from where it under-

went a recent radiation facilitated by buoyant seeds; and (3) to test whether molecular data support the origin of Pacific cycads through ancient vicariance or more recent, long-distance dispersal. The alternative scenario of ancient dispersal events is not considered, as the oldest islands in the insular South-west Pacific originated from volcanism that commenced some 40 Ma (Yan & Kroenke, 1993; Hill, 2001).

MATERIALS AND METHODS

Morphological data

Vegetative and reproductive characters were generally scored following Hill (1994b) and Hill & Stevenson (1998), unless otherwise stated. In addition to the seven taxa of subsection Rumphiae indicated in Table 2, *Cycas scratchleyana* was included for outgroup comparison (sharing non-pectinate megasporophylls but being excluded from Rumphiae by Hill, 2004). *Cycas silvestris* was also included because of similarities to subsection Rumphiae in leaf characters. Because subsection Rumphiae is thought to form a monophyletic group (Hill, 1996), most character states present in the outgroup but absent in the subsection Rumphiae were scored as plesiomorphic [0] to provide a consistent approach to character scoring (Tables 3 and 4). Phylogenetic analysis was performed using maximum parsimony with PAUP* (ver. 4; Swofford, 2002). Branch-and-bound searches were conducted using furthest taxon addition, followed by a bootstrap (BS) analysis (10,000 replicates, also using branch-and-bound) to assess confidence in individual clades.

Table 3 Character scoring for morphological characters used in the cladistic analysis.

Character number	Character	Character states
Leaves		
1	Leaf colour	0 – dark green; 1 – leaf apex yellow, when grown in sun
2	Leaf length	0 – leaves longer than 2 m; 1 – leaves shorter than 2 m
3	Percentage spinescence of petiole	0 – spines found along most of the length of the petiole; 1 – spines absent or cover generally less than 50% of petiole
4	Angle between median pinnae and rhachis	0 – less than 70°; 1 – angle generally between 70° and 80°
5	Thickness of median pinnae	0 – less than 0.30 mm thick; 1 – more than 0.30 mm thick
6	Midrib elevation	0 – strongly raised above pinna; 1 – not raised strongly above pinna
7	Mesophyll	0 – abaxial mesophyll continuous; 1 – abaxial mesophyll not continuous
Microsporophylls		
8	Shape	0 – not dorsiventrally thickened; 1 – dorsiventrally thickened
9	Cone size (male)	0 – less than 30 cm; 1 – more than 30 cm
10	Width of lamina	0 – less than 20 cm; 1 – generally more than 20 cm
11	Length of sterile apex	0 – less than 6 mm; 1 – more than 6 mm
12	Length of apical spine	0 – less than 10 mm; 1 – more than 10 mm
Megasporophylls		
13	Maximum number of ovules	0 – eight or more; 1 – no more than six
14	Length of lamina	0 – 80 cm or less; 1 – generally more than 80 cm
15	Dentation of lamina	0 – regular; 1 – irregular
16	Length of apical spine	0 – less than 15 mm; 1 – generally more than 15 mm
Seeds		
17	Size	0 – up to 50 × 70 mm; 1 – up to 30 × 40 mm
18	Spongy layer	0 – absent; 1 – present

Table 4 Data matrix of morphological characters for the species of *Cycas* studied. The characters correspond to those of Table 3 and are listed in the same order (1 to 18).

Taxon	Characters
C. bougainvilleana	00111 11110 10000 001
C. edentata	01011 11011 01101 001
*C. falcata**	00101 11??? ??10? 101
C. litoralis	01111 11011 11011 101
C. micronesica	01111 11111 00100 101
C. rumphii	00011 11111 10101 001
C. scratchleyana	00000 00000 00000 000
C. seemannii	00101 11111 10000 001
C. silvestris	01011 11000 00010 010
C. thouarsii	00001 11011 01101 001
C. zeylandica	01001 01010 00110 101
C. 'Fiji Gold'	10011 11111 10101 001

*Male cones not known (Hill, 1999).

Allozyme data

A total of 45 samples (representing the same nine taxa as used in the morphological analyses) were obtained either from cultivated plants (mostly from the Royal Botanic Garden, Sydney) or from field locations (Table 5). All taxa from *Cycas* subsection Rumphiae were sampled except for *C. falcata*, *C. litoralis* and *C. zeylanica*, which could not be obtained for the current study. Allozyme data were collected using standard starch-gel electrophoresis protocols as detailed in Keppel *et al.* (2002). Twenty-one putative loci encoded by 13 enzyme systems (Table 6) were consistently scored and used in the phenetic analysis. Nei's (1972) estimates of the genetic identity

(*I*) and genetic distance (*D*) were calculated using POPGENE (ver. 1.21; Yeh *et al.*, 1997) software, as were allele frequencies, the number of alleles per polymorphic locus (*AP*), the percentage of polymorphic loci (*P*), the observed mean heterozygosity (H_o), and the heterozygosity expected (H_e) in Hardy–Weinberg equilibrium (Nei, 1973). A phenogram was then constructed using coancestry distance (θ, Reynolds *et al.*, 1983) between species and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) procedure as implemented in the GDA software package (ver. 1.0, d12; Lewis & Zaykin, 1999). This program was preferred over others because it calculates genetic distance based on the coancestry coefficient for populations diverging by drift (Reynolds *et al.*, 1983), a likely scenario for populations of limited size on islands (Keppel *et al.*, 2002).

All alleles of variable loci were coded as either present or absent using the independent-alleles analysis (Mickevich & Mitter, 1981), which considers the loss of an allele as important as the gain of one. After testing for congruence with the morphological data set (using the ILD or partition homogeneity test in PAUP*; see Farris *et al.*, 1995), the morphological and allozyme data sets were combined for parsimony analysis using the same search parameters as used with morphological data.

RESULTS

Cladistic analysis of morphological data

The morphological data set comprised 18 variable characters (of which 11 were potentially informative) from nine taxa. Cladistic analysis of these characteristics resulted in seven most-parsimonious trees, each 26 steps long, with a

Table 5 Sources of the 45 samples used in the allozyme analysis of the present study.

Taxon	N	Collection source	Original source
Cycas bougaivilleana	2	RBG, Sydney, Australia	New Britain, New Guinea
	3	RBG, Sydney, Australia	Guadalcanal, Solomon Islands
Cycas edentata	3	RBG, Sydney, Australia	Philippines
Cycas micronesica	3	RBG, Sydney, Australia	Guam
Cycas rumphii	3	RBG, Sydney, Australia	Ambon, Halmahera, New Guinea
Cycas 'Fiji Gold'	3	Port Vila, Vanuatu	Unknown
	2	USP, Suva, Fiji	Unknown
	1	Sigatoka, Viti Levu, Fiji	Unknown
Cycas scratchleyana	1	RBG, Sydney, Australia	Ambon, Halmahera, New Guinea
Cycas seemannii	3	'Eua, Tonga	'Eua, Tonga
	3	Nabou, Viti Levu, Fiji	Nabou, Viti Levu, Fiji
	3	New Caledonia	New Caledonia
	3	Sigatoka, Viti Levu, Fiji	Naduri, Vanua Levu, Fiji
	3	Efate, Vanuatu	Efate, Vanuatu
Cycas silvestris	4	RBG, Sydney, Australia	Cape York, Australia
Cycas thouarsii	3	RBG, Sydney, Australia	Madagascar, Africa
	2	USP, Suva, Fiji	Unknown

N, number of individuals sampled; RBG, Royal Botanic Garden; USP, University of the South Pacific.

Table 6 Names of allozyme loci used in genetic analyses, presented according to IUBNC nomenclature, enzyme commission number (EC), abbreviation, and buffer system used to collect allozyme data.

IUBNC name	EC	Abbreviation	Buffer
Asparate aminotransferase	2.6.1.1	AAT-1, -2	B
Formaldehyde dehydrogenase	1.2.1.1	FDH-1	A
Fructose-biphosphate aldolase	4.1.2.13	FBA-1	D
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH-1	B
Glutamate dehydrogenase	1.4.1.2	GTDH-1	B
Isocitrate dehydrogenase	1.1.1.42	IDH-1	D
Malate dehydrogenase	1.1.1.37	MDH-1, -2, -3, -4	D
Mannose-6-phosphate isomerase	5.5.1.8	MPI-1, -2	A
Phosphoglucomutase	5.4.2.2	PGM-1	D
Phosphogluconate dehydrogenase	1.1.1.44	PGDH-1, -2	D
Shikimate dehydrogenase	1.1.1.25	SKDH-1	D
Triose-phosphate isomerase	5.3.1.1	TPI-1, -2	A
UTP-glucose-1-phosphate uridylyltransferase	2.7.7.9	UGUT-1, -2	B

IUBNC nomenclature follows Murphy *et al.* (1996). Buffer A, tris citrate/lithium borate; buffer B, citrate/sodium borate; buffer D, a modification of that buffer by Conkle *et al.* (1982); but at pH 8.

consistency index (CI, excluding autapomorphies) of 0.58 and a retention index (RI) of 0.65. The consensus of these trees is presented in Fig. 2, with bootstrap percentages. Limited outgroup sampling prevents a thorough test of monophyly for subsection Rumphiae, but support for the Rumphiae clade (i.e. all taxa excluding *C. scratchleyana* and *C. silvestris*) is strong (BS = 86%). The topology within this clade is poorly resolved, but all seven trees show a relationship between *C. edentata* and *C. thouarsii* with moderate support (BS = 67%), and suggest relationships between *C. seemannii* and *C. bougainvilleana*, and between *C. rumphii* and *C.* 'Fiji Gold' (although bootstrap support for these clades is very low).

Starch gel electrophoresis

Thirteen of the 21 putative loci were polymorphic (Table 7). Very high genetic identities (I = 0.90 to 0.95) were observed among the Pacific species (Table 8, Fig. 3), particularly between *C. rumphii* and *C. silvestris* (I = 0.9417), and between *C. thouarsii* and *C. edentata* (I = 0.9353). *Cycas rumphii* and *Cycas* 'Fiji Gold', have a genetic identity of only 0.8898, much lower than that between *Cycas* 'Fiji Gold', and *C. bougainvilleana* (I = 0.9448), *C. micronesica* (I = 0.9507) or *C. seemannii* (I = 0.9481). Within the subsection Rumphiae, there were nearly three alleles (AP = 2.69) per polymorphic locus, and more than 50% of all loci were polymorphic (P = 57.14), but heterozygosity was low ($H_o = 0.0325 \pm 0.0531$ [SD]; $H_e = 0.1749 \pm 0.2305$).
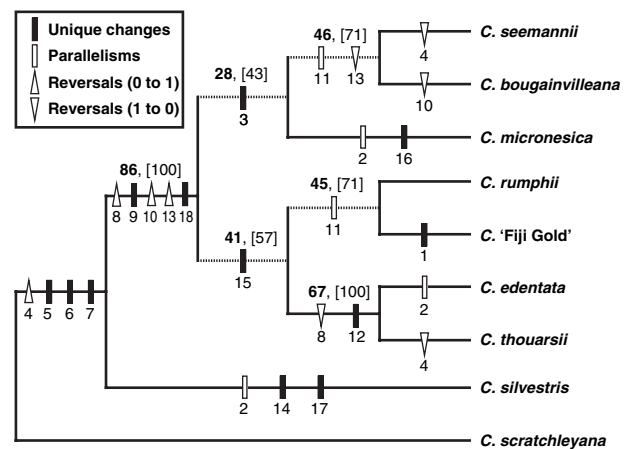

Figure 2 One of the seven shortest trees based on parsimony analysis of morphological data from *Cycas* subsection Rumphiae (CI = 0.58, RI = 0.65). Dashed nodes represent branches that collapse in the strict consensus tree; bootstrap percentages are presented above each node (in bold), followed by consensus percentages (in square brackets). Evolution of morphological characters has been reconstructed using character mapping; character numbers correspond to those in Table 4.

Combined analysis

Twenty-seven allozyme alleles were considered cladistically informative and coded as binary characters because they were variable in at least one taxon, following 'presence–absence coding' in the 'independent allele' model of Mickevich & Mitter (1981). To be considered present, an allele needed a frequency of 0.2 or more. This cut-off was implemented to avoid the disproportionate effect of rare alleles. The following alleles were scored as present (0) or absent (1): AAT-2, alleles 1 and 2; FBA-1, allele 3; IDH-1, alleles 1, 2, 3 and 5; MDH-2, alleles 1, 2 and 3; MDH-3, allele 2; PGDH-1, allele 2; PGDH-1, allele 2; PGM-1, allele 2; SKDH-1, alleles 1, 2, 3, 4 and 5; TPI-1, alleles 1 and 2; TPI-2, alleles 1 and 2; UGUT-1, allele 2; and UGUT-2, alleles 1, 2 and 3. In comparing the morphological and molecular data sets, the partition homogeneity test yielded a *P*-value of 0.274, indicating that the two data sets were not significantly incongruent and are thus combinable. After adding the 27 allozyme characters to the morphological data set, the combined data set comprised 45 characters. Cladistic analyses yielded a single most-parsimonious tree of 66 steps (CI = 0.53, excluding autapomorphies; RI = 0.59). This tree (Fig. 4) is better resolved than the consensus tree based on morphology alone, but is otherwise similar in topology, differing mostly in the placement of the *C. endentata* + *C. thouarsii* clade.

DISCUSSION

Morphological and allozyme data generally support the circumscription of *Cycas* subsection Rumphiae, which is well defined and easily distinguished by the presence of a spongy tissue in the seed (character 18) as well as by cone size (viz., exceeding 30 cm; character 9) (Fig. 2). Both the phenetic analysis of allozyme data

G. Keppel, P. D. Hodgskiss and G. M. Plunkett

Table 7 Starch gel electrophoresis allele frequencies of polymorphic loci for the *Cycas* species studied.

Locus	Allele number	Allele frequency								
		CyBo	CyEd	CySi	CyMi	CyRu	CyTh	Cy'FG'	CySc	CySe
AAT-2	1	1	1	1	1	1	1	1	0	1
	2	0	0	0	0	0	0	0	1	0
FBA-1	1	1	1	1	1	1	1	1	1	0.47
	2	0	0	0	0	0	0	0	0	0.13
	3	0	0	0	0	0	0	0	0	0.40
IDH-1	1	0.5	0	0	1	0.33	0	1	0	0.73
	2	0	0.83	0	0	0	1	0	0	0
	3	0.3	0	1	0	0.67	0	0	1	0.2
	4	0	0	0	0	0	0	0	0	0.07
	5	0.2	0	0	0	0	0	0	0	0
	6	0	0.17	0	0	0	0	0	0	0
MDH-2	1	0.50	1	1	0	0.67	0	0.93	0	0.7
	2	0	0	0	0	0	1	0	1	0
	3	0.50	0	0	1	0.33	0	0.07	0	0.30
MDH-3	1	1	1	1	1	1	1	1	1	0.80
	2	0	0	0	0	0	0	0	0	0.20
PGDH-1	1	0.3	0	0	0.83	0	0	0.86	1	0.83
	2	0.7	1	1	0.17	1	1	0.14	0	0.17
PGDH-2	1	0.6	1	1	1	1	1	1	1	1
	2	0.4	0	0	0	0	0	0	0	0
PGM-1	1	1	1	1	1	1	1	1	1	0.80
	2	0	0	0	0	0	0	0	0	0.20
SKDH-1	1	1	0	1	0.67	1	0	0	0	0.77
	2	0	1	0	0	0	1	0	0	0
	3	0	0	0	0.33	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0.20
	5	0	0	0	0	0	0	0	1	0.03
TPI-1	1	1	1	1	1	0.83	0.9	1	0	1
	2	0	0	0	0	0	0.1	0	1	0
	3	0	0	0	0	0.17	0	0	0	0
TPI-2	1	1	0.17	1	1	1	0.7	1	1	1
	2	0	0.83	0	0	0	0.3	0	0	0
UGUT-1	1	0.7	1	1	1	1	1	0.86	1	1
	2	0.3	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0.14	0	0
UGUT-2	1	0.2	0	0	0	1	0	0.14	1	1
	2	0.8	0	0	1	0	0	0.86	0	0
	3	0	1	1	0	0	1	0	0	0

CyBo, *Cycas bougainvilleana*; CyEd, *Cycas edentata*; CySi, *Cycas silvestris*; CyMi, *Cycas micronesica*; CyRu, *Cycas rumphii*; CyTh, *Cycas thouarsii*; Cy'FG', *Cycas* 'Fiji Gold'; CySc, *Cycas scratchleyana*; CySe, *Cycas seemannii*.

(Fig. 3) and the cladistic analysis of the combined data set (Fig. 4) suggest that the subsection may be further divided into two subclades. The first unites taxa from the South-west Pacific (*C. rumphii*, *C.* 'Fiji Gold', *C. seemannii*, and *C. micronesica*, to which *C. silvestris* is added in the allozyme tree), here referred to as the '*Cycas rumphii* group'. The second subclade comprises *C. edentata* and *C. thouarsii* (the '*Cycas edentata* group'), which is sister to the *C. rumphii* group in all trees except that based on morphology alone (where its placement is left unresolved; Fig. 2).

In comparing trees based on allozyme data (Fig. 3) and on the combined analysis (Fig. 4), the major difference is in the position of the Queensland endemic *C. silvestris*, which is placed as sister group to subsection Rumphiae in the cladogram (Fig. 4), but appears within the subsection Rumphiae cluster in the allozyme-based phenogram (Fig. 3). Four morphological characters unambiguously differentiate *C. silvestris* from the species of subsection Rumphiae. In addition to the diagnostic subsectional character (spongy layer in the seeds; character 18), the sizes of the sporophyll lamina (character 14), seeds (character 17), and cones (character 9) are also distinctive (Fig. 2). The surprisingly high degree of genetic identity between *C. silvestris* and *C. rumphii* ($I = 0.9353$) in particular (and to all members of subsection Rumphiae in general; $I \geq 0.8157$; Table 5) suggests a complex relationship among these taxa. The placement of *C. silvestris* in

Table 8 Nei's (1972) genetic identity (above diagonal) and genetic similarity (below diagonal) matrix. Genetic identity ranges from 0 (no genetic similarity) to 1 (genetically identical). Genetic similarity also ranges from 0 (genetically identical) to 1 (no genetic similarity).

Taxon	CyBo	CyEd	CySi	CyMi	CyRu	CyTh	CySc	Cy'FG'	CySe
CyBo	****	0.8113	0.9095	0.9429	0.9411	0.8087	0.7204	0.9448	0.9191
CyEd	0.2091	****	0.8768	0.7531	0.8277	0.9353	0.6354	0.7843	0.7894
CySi	0.0949	0.1315	****	0.8157	0.9392	0.8503	0.7143	0.8621	0.8705
CyMi	0.0588	0.2836	0.2037	****	0.8643	0.7752	0.7188	0.9507	0.9083
CyRu	0.0607	0.1891	0.0628	0.1458	****	0.8178	0.7677	0.8898	0.9417
CyTh	0.2123	0.0669	0.1621	0.2546	0.2011	****	0.7151	0.7615	0.7765
CySc	0.3279	0.4534	0.3365	0.3301	0.2644	0.3354	****	0.7297	0.7806
Cy'FG'	0.0568	0.2430	0.1484	0.0505	0.1168	0.2724	0.3151	****	0.9481
CySe	0.0844	0.2365	0.1387	0.0962	0.0601	0.2530	0.2477	0.0533	****

CyBo, *Cycas bougainvilleana*; CyEd, *Cycas edentata*; CySi, *Cycas silvestris*; CyMi, *Cycas micronesica*; CyRu, *Cycas rumphii*; CyTh, *Cycas thouarsii*; Cy'FG', *Cycas* 'Fiji Gold'; CySc, *Cycas scratchleyana*; CySe, *Cycas seemannii*.
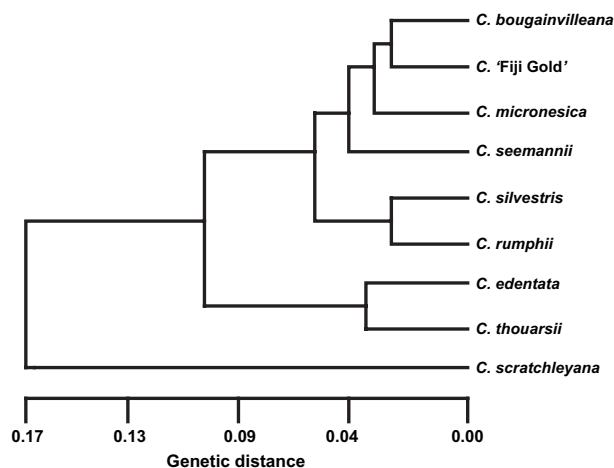

Figure 3 Phenogram showing relationships between nine taxa in the genus *Cycas*, based on distance analysis (neighbour-joining method) of allozyme data.

Figure 4 The single tree resulting from the parsimony analysis of the combined data set based on morphological and allozyme data from *Cycas* subsection Rumphiae (CI = 0.53, RI = 0.59). Bootstrap percentages are presented above each node. Geographic ranges are provided next to each taxon name.

Fig. 4 (as the sister group to subsection Rumphiae), if correct, suggests that this lineage diverged from the Rumphiae lineage prior to the evolution of the spongy seed layer. The alternative explanation, that of the placement of *C. silvestris* within subsection Rumphiae (as indicated in the allozyme phenogram; Fig. 3), would necessitate the secondary loss, after a dispersal event, not only of the diagnostic spongy layer but also of characters 9, 14, and 17, which is an unparsimonious scenario that would also imply that the subsection (as currently circumscribed) is paraphyletic. In fact, the allozyme topology (Fig. 3) is nine steps longer than the most-parsimonious solutions of both the morphological and the combined data set.

Another scenario that could account for the placement of the species is hybridization between *C. silvestris* and *C. rumphii* (or their ancestors). This possibility is made more likely by the geographic proximity of the two species in far-northern Queensland (*C. silvestris*) and nearby eastern New Guinea (*C. rumphii*). In addition, members in the genus *Cycas* generally hybridize freely. For example, Hill (1994b) men-
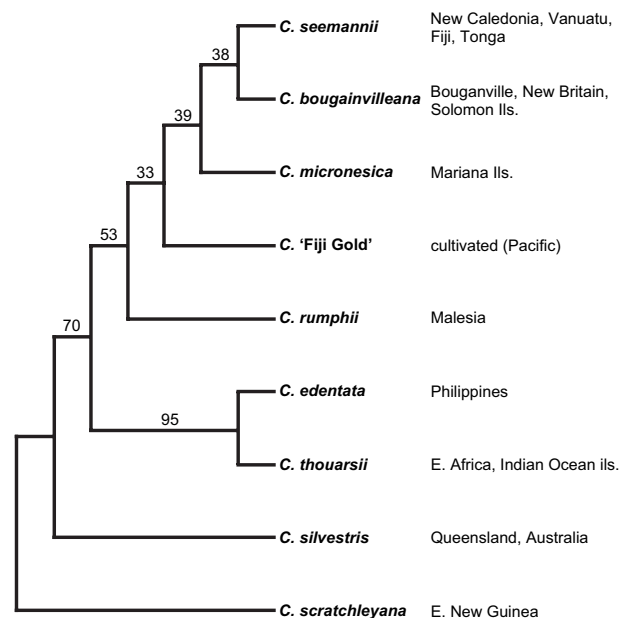
tioned putative hybridization of *C. bougainvilleana* with *C. seemannii*, *C. scratchleyana* and *C. rumphii*. Additional molecular data may help to test this hypothesis.

Morphological data (either alone or in combination with allozyme characters) fail to resolve definitively the placement of the 'yellow cycad' (*Cycas* 'Fiji Gold'). Fig. 2, however, suggests a possible relationship to *C. rumphii*, but only in some of the most-parsimonious trees (five of the seven trees based on morphology alone, and two of the five trees based on the combined data). This result would be consistent with the designation of the yellow cycad as '*C. rumphii* f. *aurea*', but not with its position in the phenogram based on allozyme data, nor with its position in the combined analysis. In the phenogram

(Fig. 3), the yellow cycad clusters with *C. bougainvilleana* in the Pacific island group, whereas in the combined tree it is placed in a grade with *C. rumphii* at the base of the clade that unites *C. seemannii*, *C. bougainvilleana* and *C. micronesica*. As with *C. silvestris*, the explanation for these discrepancies may be interspecific hybridization. However, no natural populations of yellow cycads have been discovered, although they could possibly exist in Vanuatu or the Santa Cruz archipelago of the Solomon Islands.

Although we cannot comment on the genetic diversity within the various species because of the small sample sizes, our data confirm the general trend towards low heterozygosity in populations in many cycads (Appendix 2 in González-Astorga *et al.*, 2006). Genetic drift in relatively small populations resulting from few colonizers (the founder effect) is a likely contributing factor. Inbreeding as a result of limited seed and pollen dispersal distances is another potentially important factor. New molecular techniques that can infer seed and pollen dispersal distances from fine-scale spatial genetic structure (Degen *et al.*, 2001) could be used to investigate the importance of this.

The genetic identities found within subsection Rumphiae are higher than the interspecific identity ($I = 0.67 \pm 0.07$) proposed by Gottlieb (1977). Only the outgroup, *C. scratchleyana*, is differentiated at this level from members of the subsection Rumphiae ($I = 0.64–0.78$). The *Cycas edentata* group is differentiated from the *Cycas rumphii* group at lower levels ($I = 0.72–0.82$), and species within these groups are even more similar ($I = 0.86–0.95$), falling within the range proposed for conspecific populations ($I = 0.95 \pm 0.02$; Gottlieb, 1977). Low genetic distances between morphologically distinct species are common in cycads and have been attributed to recent speciation (Yang & Meerow, 1996; van der Bank *et al.*, 2001; Sharma *et al.*, 2004). In the Pacific, the distribution of the subsection Rumphiae across geologically young islands of the South-west Pacific is almost certainly a result of relatively recent dispersal events, which may be ongoing. This supports Hill's (1996) hypothesis of a recent colonization of the Pacific by means of long-distance and probably oceanic stepping-stone events. Seed buoyancy probably accounts for such recent and rapid radiation (particularly for slow-growing, woody species) and the higher-than-expected genetic identities found among species that are morphologically distinct.

The close genetic relationship among three of the Pacific island species suggests that *C. bougainvilleana*, *C. micronesica* and *C. seemannii* share a common ancestry. All species found outside this group (e.g. *C. rumphii*, *C. edentata*) are centred in or around Malesia, suggesting that this region served as the source area for dispersals to the Pacific, Africa and possibly Australia. Our data do not allow us to discern the particular sequence of dispersal. It is possible, for example, that there were several independent dispersal events from Malesia to each group of Pacific islands (north-east Malesia for *C. bougainvilleana*, south-east Malesia for *C. seemannii*, and Micronesia for *C. micronesica*). Alternatively, dispersal may have followed a stepping-stone model from the source area to island groups successively farther east, for which several scenarios are possible. Among the possibilities would be dispersal from Malesia to Micronesia, thence to north-east Melanesia, and finally to south-east Melanesia (consistent with the morphological tree) or to south-east Melanesia, Micronesia, and north-east Melanesia (more consistent with the allozyme tree).

This recent radiation of the old *Cycas* lineage, and other apparently recent speciation events in cycads (Yang & Meerow, 1996; van der Bank *et al.*, 2001; Sharma *et al.*, 2004) show that many cycad species are not relics but have recently evolved. It also implies that evolutionarily old taxa are not necessarily relics of formerly wider distributions. Relatively recent diversification of an old lineage has also been observed for the conifer genus *Agathis*, which has colonized the Fiji, Santa Cruz and Vanuatu archipelagos from New Caledonia (Setoguchi *et al.*, 1998). Therefore, the presence of old lineages in a particular area or on an island should not be used to infer the antiquity of that area/island or its vegetation.

Future studies are needed to test many of the alternative scenarios proposed herein, but the present study demonstrates that starch gel electrophoresis (together with morphological data) can provide useful information for investigating relationships among the species of *Cycas*. These findings reinforce results from earlier electrophoretic studies of two other cycad genera, *Encephalartos* (van der Bank *et al.*, 1998) and *Macrozamia* (Sharma *et al.*, 1998). However, the uncertainty in the placement of certain taxa also highlights the limitations of starch gel electrophoresis. Other molecular markers that are potentially more variable, such as amplified fragment length polymorphisms (AFLPs), microsatellites, and random amplified polymorphic DNA (RAPD), could be used to obtain additional information. For example, DNA sequences obtained from organelles revealed much genetic variation in *C. taitungensis*, which had shown very low allozymic variation (Huang *et al.*, 2001). Given the known utility of molecular techniques in elucidating patterns of hybridization, future studies may seek to resolve the placements of both *Cycas* 'Fiji Gold' and *C. silvestris*, in which hybridization may have played a role.

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REFERENCES

van Balgooy, M.M.J. (1969) A study on the diversity of island floras. *Blumea*, **17**, 139–178.

van Balgooy, M.M.J. (1996) Phytogeography of the Pacific – floristic and historical distribution patterns in plants. *The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: patterns and processes* (ed. by A. Keast and S.E. Miller), pp. 191–213. SPB Academic Publishing, Amsterdam.

van der Bank, F.H., Vorster, P. & van der Bank, M. (1998) Phylogenetic relationships, based on allozyme data, between six cycad taxa indigenous to South Africa. *South African Journal of Botany*, **64**, 182–188.

van der Bank, F.H., Wink, M., Vorster, P., Treutlein, J., Brand, L., van der Bank, M. & Hurter, J. (2001) Allozyme and DNA sequence comparisons of nine species of *Encephalartos* (Zamiaceae). *Biochemical Systematics and Ecology*, **29**, 241–266.

Bremer, B., Andreasen, K. & Olsson, S. (1995) Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden*, **82**, 383–397.

Chaw, S.-M., Walters, T.W., Chang, C.-C., Hu, S.-H. & Chen, C.-H. (2005) A phylogeny of cycads (Cycadales) inferred from chloroplast *matK* gene, *trnK* intron, and nuclear rDNA ITS region. *Molecular Phylogenetics and Evolution*, **37**, 214–234.

Conkle, M.T., Hodgskiss, P.D., Nunally, L.B. & Hunter, S.C. (1982) *Starch gel electrophoresis of conifer seeds: a laboratory manual*. Pacific Southwest Forest and Range Experiment Station, Berkeley, CA.

Corner, E.J.H. (1963) *Ficus* in the Pacific Region. *Pacific Basin biogeography – a symposium* (ed. by J.L. Gressit), pp. 233–245. Bishop Museum Press, Honolulu, Hawaii.

Crawford, D.J. (1983) Phylogenetic and systematic inferences from electrophoretic studies. *Isozymes in plant genetics and breeding, part A* (ed. by S.D. Tanksley and T.J. Orton), pp. 257–287. Elsevier Science Publishers, Amsterdam.

Crawford, D.J. (1989) Enzyme electrophoresis and plant systematics. *Isozymes in plant biology* (ed. by D.E. Soltis and P.S. Soltis), pp. 146–164. Dioscorides Press, Portland, OR.

Degen, B., Caron, H., Bandou, E., Maggia, L., Chevallier, H., Leveau, A. & Kremer, A. (2001) Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity*, **87**, 497–507.

Dehgan, B. & Yuen, C.K.K.H. (1983) Seed morphology in relation to dispersal, evolution and propagation of *Cycas* L. *Botanical Gazzette*, **144**, 412–418.

DeJoode, D.R. & Wendel, J.F. (1992) Genetic diversity and origin of the Hawaiian island cotton, *Gossypium tomentosum*. *American Journal of Botany*, **79**, 1311–1319.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1995) Testing significance of incongruence. *Cladistics*, **10**, 315–319.

González-Astorga, J., Vovides, A.P., Octavio-Aguilar, P., Aguirre-Fey, D., Nicolalde-Morejón, F. & Iglesias, C. (2006) Genetic diversity and structure of the cycad *Zamia loddigesii* Miq. (Zamiaceae): implications for evolution and conservation. *Botanical Journal of the Linnean Society*, **152**, 533–544.

Gottlieb, L.D. (1977) Electrophoretic evidence and plant systematics. *Annals of the Missouri Botanical Garden*, **64**, 161–180.

Heads, M. (2006) Seed plants of Fiji: an ecological analysis. *Biological Journal of the Linnean Society*, **89**, 407–431.

Hill, K.D. (1994a) The *Cycas media* group (Cycadaceae) in New Guinea. *Australian Systematic Botany*, **7**, 527–541.

Hill, K.D. (1994b) The *Cycas rumphii* complex (Cycadaceae) in New Guinea and the Western Pacific. *Australian Systematic Botany*, **7**, 543–567.

Hill, K.D. (1994c) The golden cycad of Fiji. *Encephalartos*, **38**, 11–13.

Hill, K.D. (1995) Infrageneric relationships, phylogeny, and biogeography of the genus *Cycas* (Cycadaceae). *Proceedings of the Third International Conference of Cycad Biology* (ed. by P. Vorster), pp. 139–162. Cycad Society of South Africa, Stellenbosch.

Hill, K.D. (1996) Cycads in the Pacific. *The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: patterns and processes* (ed. by A. Keast and S.E. Miller), pp. 267–274. SPB Academic Publishing, Amsterdam.

Hill, K.D. (1999) *Cycas falcata* (Cycadaceae), a new species from Sulawesi, Indonesia. *Kew Bulletin*, **54**, 209–213.

Hill, K.D. (2004) Character evolution, species recognition and classification concepts in the Cycadaceae. *Cycad classification: concepts and recommendations* (ed. by T. Walters and R. Osborne), pp. 23–44. CABI Publishing, Wallingford.

Hill, K.D. & Stevenson, D.W. (1998) *The Cycad Pages*. http://plantnet.rbgsyd.gov.au/PlantNet/cycad.

Hill, K.D. & Yang, S.-L. (1999) The genus *Cycas* (Cycadaceae) in Thailand. *Brittonia*, **51**, 48–73.

Hill, K.D., Stevenson, D.W. & Osborne, R. (2007) The world list of cycads (La lista mundial cicadas). *Memoirs of the New York Botanical Garden*, **97**, 454–483.

Hill, R. (2001) Cenozoic reconstruction of SE Asia and the SW Pacific: changing patterns of land and sea. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J.M.B. Smith, M. Morwood and I.D. Davidson), pp. 35–56. A.A. Balkema (Swets & Zeitlinger Publishers), Lisse.

Huang, S., Chiang, Y.C., Schaal, B.A., Chou, C.-H. & Chiang, T.Y. (2001) Organelle DNA phylogeography of *Cycas taitungensis*, a relict species in Taiwan. *Molecular Ecology*, **10**, 2669–2681.

Jordan, G.J. (2001) An investigation of long-distance dispersal based on species native to both Tasmania and New Zealand. *Australian Journal of Botany*, **49**, 333–340.

Keppel, G. (2001) Notes on the natural history of *Cycas seemannii* (Cycadaceae). *South Pacific Journal of Natural Sciences*, **19**, 35–42.

Keppel, G., Lee, S.-W. & Hodgskiss, P.D. (2002) Evidence for long isolation among populations of a Pacific cycad: Genetic diversity and differentiation in *Cycas seemannii* A. Br. (Cycadaceae). *Heredity*, **93**, 133–139.

Kim, H.-G., Keeley, S.C., Vroom, P.S. & Jansen, R.K. (1998) Molecular evidence for an African origin of the Hawaiian endemic *Hesperomannia* (Asteraceae). *Proceedings of the National Academy of Sciences USA*, **95**, 15440–15445.

Knox, E.B. & Palmer, J.D. (1995) The origin of *Dendro-senecio* within the Senecioneae (Asteraceae) based on chloroplast DNA evidence. *American Journal of Botany*, **82**, 1567–1573.

de Laubenfels, J.D. (1996) Gondwanan conifers on the Pacific Rim. *The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: patterns and processes* (ed. by A. Keast and S.E. Miller), pp. 261–265. SPB Academic Publishing, Amsterdam.

de Laubenfels, J.D. & Adema, F. (1998) A taxonomic revision of the genera *Cycas* and *Epicycas* gen. nov. (Cycadaceae). *Blumea*, **43**, 351–400.

Lewis, P.O. & Zaykin, D. (1999) *Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.0 (d12)*. Free program distributed by the authors over the internet from the GDA Home Page at http://chee.unm.edu/gda/.

Lindqvist, C. & Albert, V.A. (2002) Origin of the Hawaiian endemic mints with North American *Stachys* (Lamiaceae). *American Journal of Botany*, **89**, 1709–1724.

Lindström, A.J. & Hill, K.D. (2002) Notes on species of *Cycas* (Cycadaceae) from Sri Lanka and islands of the Andaman Sea. *Novon*, **12**, 237–240.

Mickevich, M.F. & Mitter, C. (1981) Treating polymorphic characters in systematics: a phylogenetic treatment of electrophoretic data. *Advances in cladistics. Proceedings of the first meeting of the Willi Henning Society* (ed. by V.A. Funk and D.R. Brooks), pp. 45–58. New York Botanical Garden, Bronx, NY.

Motley, T.J., Wurdack, K.J. & Delprete, P.G. (2005) Molecular systematics of the Catesbaeeae–Chiococceae complex (Rubiaceae): flower and fruit evolution and biogeographic implications. *American Journal of Botany*, **92**, 316–329.

Murphy, R.W., Sites, J.W., Jr, Buth, D.G. & Haufler, C.H. (1996) Proteins: isozyme electrophoresis. *Molecular systematics* (ed. by D.M. Hillis, C. Moritz and B.K. Mabel), pp. 51–120. Sinauer Associates, Sunderland, MA.

Nei, M. (1972) Genetic distance between populations. *The American Naturalist*, **106**, 283–292.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA*, **70**, 3321–3323.

Newell, S.J. (1989) Intrapopulational variation in leaflet morphology among populations of Caribbean cycads (*Zamia*). *American Journal of Botany*, **76**, 1518–1523.

Norstog, K.J. & Nicholls, T.J. (1997) *The biology of cycads*. Cornell University Press, Ithaca, NY.

Pole, M.S. (1994) The New Zealand flora – entirely long-distance dispersal? *Journal of Biogeography*, **21**, 625–635.

Pole, M.S. (2001) Can long-distance dispersal be inferred from the New Zealand plant fossil record? *Australian Journal of Botany*, **49**, 357–366.

Reynolds, J., Weir, B.S. & Cockerham, C.C. (1983) Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.

Schuster, J. (1932) Cycadaceae. *Das Pflanzenreich*, **99**, 1–168.

Seelanan, T., Schnabel, A. & Wendel, J.F. (1997) Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany*, **22**, 259–290.

Setoguchi, H., Osawa, T.A., Pintaud, J.-C., Jaffré, T. & Veillon, J.-M. (1998) Phylogenetic relationships within Araucariaceae based on *rbcL* gene sequences. *American Journal of Botany*, **85**, 1507–1516.

Sharma, I.K., Jones, D.L., Forster, P.I. & Young, A.G. (1998) The extent and structure of genetic variation in the *Macrozamia pauli-guiliemi* complex (Zamiaceae). *Biochemical Systematics and Ecology*, **26**, 45–54.

Sharma, I.K., Jones, D.L. & Forster, P.I. (2004) Genetic differentiation and phenetic relatedness among seven species of the *Macrozamia plurinervia* complex (Zamiaceae). *Biochemical Systematics and Evolution*, **32**, 313–327.

Smitinand, T. (1971) The genus *Cycas* Linn. (Cycadaceae) in Thailand. *Natural History Bulletin of the Siam Society*, **14**, 163–175.

Soltis, D.E., Soltis, P.S., Endress, P.K. & Chase, M.W. (2005) *Phylogeny and evolution of angiosperms*. Sinauer Associates, Sunderland, MA.

van Steenis, C.G.G.J. (1962) The land-bridge theory in botany. *Blumea*, **11**, 235–542.

Swofford, D.L. (2002) *PAUP*: Phylogenetic analysis using parsimony (*and other methods): version 4*. Sinauer Associates, Sunderland, MA.

Thorne, R.F. (1963) Biotic distribution patterns in the tropical Pacific. *Pacific basin biogeography* (ed. by J.L. Gressit), pp. 311–350. Bishop Museum Press, Honolulu, Hawaii.

Vorster, P. (1993) Taxonomy of *Encephalartos* (Zamiaceae): taxonomically useful external characteristics. *Proceedings of CYCAD 90, the Second International Conference on Cycad Biology* (ed. by D.W. Stevenson and K.J. Norstog), pp. 294–299. Palm and Cycad Societies of Australia Ltd, Milton, Queensland.

Wang, D.Y. (1996) Systematic classification and brief introduction to Cycadales (Chapter 2), Taxonomy of *Cycas* in China (Chapter 3). *Cycads in China* (ed. by F.X. Wang and H.B. Liang), pp. 9–142. Guangdong Science and Technology Press, Guangdong.

Weeden, N.F. & Wendel, J.F. (1989) Genetics of plant isozymes. *Isozymes in plant biology* (ed. by D.E. Soltis and P.S. Soltis), pp. 47–71. Dioscorides Press, Portland, OR.

Weston, P.H. & Crisp, M.D. (1996) Trans-Pacific biogeographic patterns in the Proteaceae. *The origin and evolution of Pacific island biotas, New Guinea to Eastern Polynesia: patterns and processes* (ed. by A. Keast and S.E. Miller), pp. 215–232. SPB Academic Publishing, Amsterdam.

Whitmore, T.C. (1969) The vegetation of the Solomon Islands. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **255**, 259–270.

Whitmore, T.C. (1980) A monograph of *Agathis*. *Plant Systematics and Evolution*, **135**, 41–69.

Whittaker, R.J., Bush, M.B. & Richards, K. (1989) Plant recolonization and vegetation succession on the Krakatau Islands, Indonesia. *Ecological Monographs*, **59**, 59–123.

Winkworth, R.C., Wagstaff, S.J., Glenny, D. & Lockhart, P.J. (2002) Plant dispersal N.E.W.S. from New Zealand. *Trends in Ecology and Evolution*, **17**, 514–520.

Woodroffe, C.D. (1987) Pacific Island mangroves: distribution and environmental settings. *Pacific Science*, **41**, 166–185.

Wright, S.D., Yong, C.G., Dawson, J.W., Whittaker, D.J. & Gardner, R.C. (2000) Riding the ice age El Niño? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros* (Myrtaceae) inferred from nuclear ribosomal DNA. *Proceedings of the National Academy of Sciences USA*, **97**, 4118–4123.

Yan, C.Y. & Kroenke, L.W. (1993) A plate tectonic reconstruction of the Southwest Pacific, 0–100 Ma. *Proceedings of the Ocean Drilling Program, scientific results*, Vol. 130 (ed. by W.H. Berger, L.W. Kroenke, T.R. Janecek, J. Backman, F. Bassinot, R.M. Corfield, M.L. Delaney, R. Hagen, E. Jansen, L.A. Krissek, C. Lange, R.M. Leckie, I.L. Lind, M.W. Lyle, J.J. Mahoney, J.C. Marsters, L. Mayer, D.C. Mosher, R. Musgrave, M.L. Prentice, J.M. Resig, H. Schmidt, R. Stax, M. Storey, K. Takahashi, T. Takayama, J.A. Tarduno, R.H. Wilkens & G. Wu), pp. 697–709. Ocean Drilling Program, College Station.

Yang, S.-L. & Meerow, A.W. (1996) The *Cycas pectinata* (Cycadaceae) complex: genetic structure and gene flow. *International Journal of Plant Science*, **157**, 468–483.

Yeh, F.C., Yang, R.-C., Boyle, T.J.B., Ye, Z.-H. & Mao, J.X. (1997) *POPGENE, the user-friendly shareware for population genetic analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton.

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